The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subj[illegible] the company to certain additional company law rules.

Signed [signature]

Dated 23 October 2003

PRIORITY DOCUMENT

SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

Pate(Form 1/77

Patents Act 1977
(Rule 16)

The Patent Office

10OCT02 E754829-1 D01891
P01/7700 0.00-0223562.0

THE PATENT OFFICE
A
10 OCT 2002
RECEIVED BY FAX

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office

Cardiff Road
Newport
Gwent NP9 1RH

1. Your reference	P1340
2. Patent application number *(The Patent Office will fill in this part)*	0223562.0
3. Full name, address and postcode of the or of each applicant *(underline all surnames)*	THE SECRETARY OF STATE FOR DEFENCE DSTL Porton Down Salisbury Wiltshire. SP4 0JQ. UK
Patents ADP number *(if you know it)*	6997670005
If the applicant is a corporate body, give the country/state of its incorporation	GB
4. Title of the invention	APPARATUS FOR MOVING PARTICLES
5. Name of your agent *(if you have one)*	Beckham Robert Willaim
"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*	D/IPR Formalities Section Poplar 2 MoD Abbey Wood #2218 Bristol BS34 8JH
Patents ADP number *(if you know it)*	8281404001

	Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*
6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number			

	Number or earlier application	Date of filing *(day / month / year)*
7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application		

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:* *a) any applicant named in part 3 is not an inventor, or* *b) there is an inventor who is not named as an applicant, or* *c) any named applicant is a corporate body.* *See note (d))*	Yes

0049533 10-Oct-02 04:44

Pat[illegible] Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	
Description	4
Claim(s)	2
Abstract	1
Drawing(s)	3

10. If you are also filing any of the following, state how many against each item.

Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	One
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. I / We request the grant of a patent on the basis of this application.

Signature R.B. Luetchford Date 10/10/02

RB Luetchford

12. Name and daytime telephone number of person to contact in the United Kingdom Mrs JM Northcott 0117 91 32860

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent of the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

a) If you need help to fill in this form or have any questions, please contact the Patent Office on 0645 500505.

b) Write your answers in capital letters using black ink or you may type them.

c) If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.

d) If you have attached 'Yes' Patents Form 7/77 will need to be filed.

e) Once you have filled in the form you must remember to sign and date it.

f) For details of the fee and ways to pay please contact the Patent Office.

DUPLICATE

APPARATUS FOR MOVING PARTICLES

1 Introduction

Ultrasound standing wave radiation acts on suspensions and colloids to drive the phases (particles, bubbles, oil droplets and their suspending phase) towards either nodal or anti-nodal positions. Laminar flow provides a stable environment free from turbulence this condition allows the radiation positioned phases to be freely manipulated downstream from the force field. Standing wave radiation and laminar flow have been used separately and together to form systems with a number of novel properties.

These are active systems with no moving mechanical parts or consumable components. They are appropriate for complex automation tasks, micro-fluidic systems and use in inaccessible locations.

When compared to a standard membrane type filter, standing wave filters have the following advantages. The channel has negligible backpressures due to its large cross-section, 0.25 x 10 mm. The design is a simple dividing channel not easily blocked. The force acting on the particles is gentle < a centrifuge running at 100 g. An exposure time of < 1 s is required to reduce the concentration of 5 μm diameter latex particles in water by 1000 fold.

The non-turbulent properties of laminar flow have been used to directly transfer particles from one medium to another (an FFF type approach). This form of particle washing takes < 1 s and removes the need for the usual concentration step .

Particle manipulation by standing wave radiation can be used to reduce diffusion barriers in immuno-tests and other particle contact reactions systems. Particles can be concentrated at chosen positions within the chamber or at the chamber wall

A high efficiency analytical scale filter based on the combination of ultrasound standing waves and laminar flow has previously been described [1] and good agreement has been found between detailed modelling of sound energy transfer to the liquid layer and the filters measured efficiency [2, 3]. The three critical features of this filter are

1) *Production of an ultrasonic standing wave radiation force which separates dissimilar phases to the nodal and anti-nodal positions*. For example, bacteria in an aqueous medium are driven towards the pressure node while air bubbles in an aqueous medium are driven towards the pressure anti-node.
2) *Sub-wavelength thickness of the liquid layer*. Unlike multi-wavelength filters [4, 5], this produces a controlled single band of particles, often a core feature for particle manipulation [6, 7].

3) *Laminar flow conditions throughout the system* provide an additional mechanism of fluid manipulation, and fewer variables than turbulent systems.

We have previously described the use of similar features in a commercially available immuno agglutination device [8] (Immunosonic, Electro Medical Supplies, Wantage, UK). One early evolution of the filter incorporating all its critical features is a device to position particles within a gel [9]. These same features are used in a continuous flow medium exchange system described here, in which particles are transferred from one medium to another without the need for a concentration (pellet formation) step, as required during centrifugal washing procedures.

1.1 Particle washing; principle of operation

The system can be described as a field-flow fractionation (FFF) type system [10, 11], the field used is an ultrasound standing wave. Two fluids (the first a suspension of particles < 50 % of the flow volume, the second a particle-free receiving fluid > 50 % of the flow volume) run in contact with each other without mixing through a 0.25 mm x 10 mm cross section duct (shown schematically in Figure 1). The two fluids pass through a standing wave field that moves the particles to the centre of the duct transferring them from fluids 1 to 2. After transfer the particles and their initial host fluid are removed through separate outlets.

2 Methods

2.1 Sample preparations

Degassed water was produced by boiling water for 2 min, sealing the water in a screw cap bottle with no air gap and leaving it to cool to room temperature before use. Dried yeast (Boots, Nottingham, UK) was reconstituted and the cells suspended at a concentration of 1×10^{8} ml^{-1} in degassed water containing 1 % (v/v) red food colouring (Carmoisine, Sunset Yellow, citric acid, preservative) (Supercook, Sherburn-in-Elmet, Leeds, UK).

2.2 Flow system

Figure 2 shows the internal ducts of the particle washing system. (0.25 x 10 mm cross section of all ducts). This has two inlets (A and B), which meet at right angles, their fluids converge and pass along a single duct (60 mm long) where they are exposed to the sound field (20 mm long). The fluid is then divided and leaves through two outlet ducts (C and D).

Fluid was pumped using three pumps (Gilson Mini-puls 3) fitted with air damped pulse smoothing consisting of pipettes and narrow tubing described previously [1]. The pumps were placed on outlet C (3.66 $ml.min^{-1}$) outlet D (0.99 $ml.min^{-1}$) and inlet B (0.56 $ml.min^{-1}$). Inlet A was left open (in flow 4.09 $ml.min^{-1}$). The total volume flow rate in the system was 4.65 $ml.min^{-1}$ (through the sound field). The Reynolds number for this region is 8.6, therefore flow is entirely laminar throughout the system. The interface between the 12 % of the total flow volume entering inlet B and the 88 % entering inlet A is found (when the parabolic flow profile is taken into account) to be approximately 53 μm from the wall.

2.3 Chamber construction details

The chamber is a modification of a previous construction [1]. The 10 mm wide walls of the channel are formed from a stainless steel (Stavax) ultrasound reflector 1.5 mm thick (3/4 wavelengths at 3 MHz) and a stainless steel sound transmission layer 2.5 mm thick (5/4 wavelengths at 3 MHz). The 0.25 mm walls are formed from a silicone rubber gasket around the 10 mm wide channel. This narrow dimension is defined, when the parts are clamped together, by a 0.25 mm thick brass shim around the gasket.

The stainless steel transmission layer contains two 0.25 x 10 mm slots, 60 mm apart each cut at right angles to the inner face of the channel. The lower slot formed a fluid converging inlet, the upper slot a fluid diverging outlet (B and D in Figure 1). The silver electrodes on PZ26 piezo-ceramic plate (Ferroperm, Krisgard, Denmark) 30 x 30 x 0.67 mm (i.e. 3 MHz fundamental thickness resonance) were etched to reduce the active area to 10 x 20 mm and attached by epoxy resin to the air face of the transmission layer half way between slots B and D.

2.4 Electrical drive

A voltage of 2.5 V was applied to the piezo-ceramic at a frequency in the region of 2.91 MHz, this frequency was a current / voltage phase minimum. Phase measurements were made with the phase comparator block of a Phase-locked loop IC (Philips PC74HC4046AP). During operation this phase minimum was tracked as previously described for voltage minimum tracking systems [5]. There is a small frequency difference between the voltage and phase minima and the system is near resonance at both frequencies. However the electrical phase minimum frequency represents the acoustic resonance more accurately than the voltage minimum.

3 Results and Discussion

3.1 Flow separation

After fluids entering inlets A and B converge they flow along the same channel, before being separately drawn from outlets C and D, see Figure 3a. In order to obtain this visually clear output from outlet C its outflow was reduced to 10.5 % below the inflow to inlet A.

In this system some of its properties which cannot easily be measured have been calculated. Turbulent mixing need not be considered for this low Reynolds number system. Therefore, assuming a parabolic flow profile, the interface between the liquids forms at 53 μm from the channel wall, at which distance flow velocity is 31 mm s^{-1}. Thus contact lasts 1.9 s as the fluids flow 60 mm along the channel (shown in Figure 1). A remaining mixing force in non-turbulent systems is diffusion i.e. dialysis can occur across the interface. The average distance (l) that a particle will diffuse in time t is obtained from Einstein's relation for diffusion of small spheres in a viscous fluid $l = \sqrt{tD}$ [12], where D (diffusion coefficient) $= kT/6\pi r\eta$ (k is Boltzmann's constant, T temperature and η the medium viscosity). From this

we find that during the 1.9 s contact between the aqueous fluids yeast cells of 2.5 μm radius (r) [5] will diffuse on average 0.4 μm, and 0.5 nm radius particles (estimated size of dye molecule) will diffuse 30 μm. Therefore diffusion would not be a significant cause of mixing for the 2.5 μm radius particles and will produce only a low percentage of mixing of 0.5 nm radius molecules. This mixing level may partially account for the need to reduce flow-rate from outlet C to a level 10 % below the flow-rate into inlet A in order to maintain a visually clear output, although engineering precision and residual pump pulses are probably of greater significance.

3.2 The effect of ultrasound

When the ultrasound was turned on the particles emerged from outlet C without visible carry over of red dye. The red dye leaving outlet D became depleted of particles.

The ultrasound radiation force selectively moves yeast cells rather than dye towards the central node position in the chamber, since the velocity component towards the node, increases with the square of the particle radius [5] (i.e. the yeast cell moves 2.5×10^7 times faster than the dye molecule).

This continuous washing process could be maintained indefinitely provided the ultrasound power was kept low. The standing wave radiation force acting to move the particles to the node is dominant in this system, however ultrasound puts many other streaming forces on particles. In a half wavelength chamber Rayleigh streaming is the most important of these forces [13]. The use of higher ultrasound voltage (power) levels did produce some carry over of dye (data not shown). It has not been determined whether this is due to streaming or small amounts of dye moving with the particle, which could diffuse away from slower moving particles.

CLAIMS

1. Apparatus for moving particles entrained in a first fluid to a second fluid, comprising a conduit, means providing for contacting laminar flow of each fluid within the conduit and means capable of generating a standing sound wave having a pressure node disposed within the conduit.

2. Apparatus according to Claim 1, in which the means providing for contacting laminar flow minimise mixing between the two fluids.

3. Apparatus according to Claim 1 or Claim 2, in which the means for arranging contacting laminar flow comprise respective inlet and outlet means for each fluid in communication with the conduit.

4. Apparatus according to Claim 3, in which the respective inlet and outlet means are orthogonal to each other.

5. Apparatus according to any preceding Claim, in which the pressure node is centrally disposed along the longitudinal length of the conduit.

5. Apparatus according any preceding Claim, in which the means capable of generating the standing sound wave comprise a first wall of the conduit adapted to generate and transmit a sound wave and a second, opposite wall adapted to reflect the generated sound wave.

6. Apparatus according to Claim 5, in which the first wall of the conduit comprises a piezoceramic material.

7. Apparatus according to any preceding Claim, in which the sound wave is an ultrasound wave.

8. A method of moving particles entrained in a first fluid to a second fluid, comprising the steps of i) providing for contacting laminar flow of each fluid within a conduit having means capable of generating a standing sound wave and ii) generating a standing wave having a pressure node within the conduit.

9. A method of washing particles according to Claim 8.

10. Use of apparatus according to Claims 1 to 7 for washing particles.

11. Apparatus substantially as hereinbefore described with reference to and as shown in the accompanying drawings.

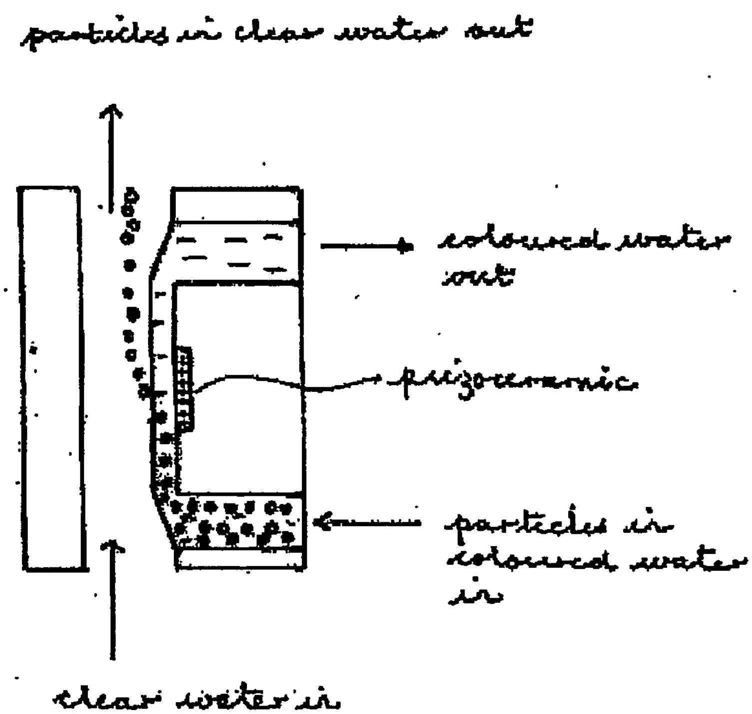
12. A method for washing particles substantially as hereinbefore described with reference to and as shown in the accompanying drawings.

Abstract:

Standing wave radiation and laminar flow have been used to directly transfer particles from one medium to another (washing) by a field-flow fractionation (FFF) approach. Ultrasound standing wave radiation acts on suspensions and colloids to drive the phases (particles/ bubbles/ oil droplets, and their suspending phase) towards either nodal or anti-nodal positions. Laminar flow provides a stable environment free from turbulence; this condition allows the radiation-positioned phases to be freely manipulated downstream from the force field. Particle washing takes < 1 s and removes the need for pellet formation and resuspension steps of centrifuge based washing procedures. Similar to a filter previously described, the ultrasound operates at approximately 3 MHz and forms a single band of particles at the centre of the chamber. With no moving mechanical parts or consumable components, it is appropriate for complex automation tasks, micro-fluidic systems, and use in inaccessible locations. The channel has negligible backpressures (cross-section, 0.25 x 10 mm). The design is a simple dividing channel that is not easily blocked. The force acting on the particles is small, less than that of a centrifuge running at 100 g. The exposure time to ultrasound is < 1 s.

1/3

MODE: SOUND ON



o particle ; – dye solution ; + piezoceramic

Figure I. Schematic representation of ultrasonic particle washing chamber.

2/3

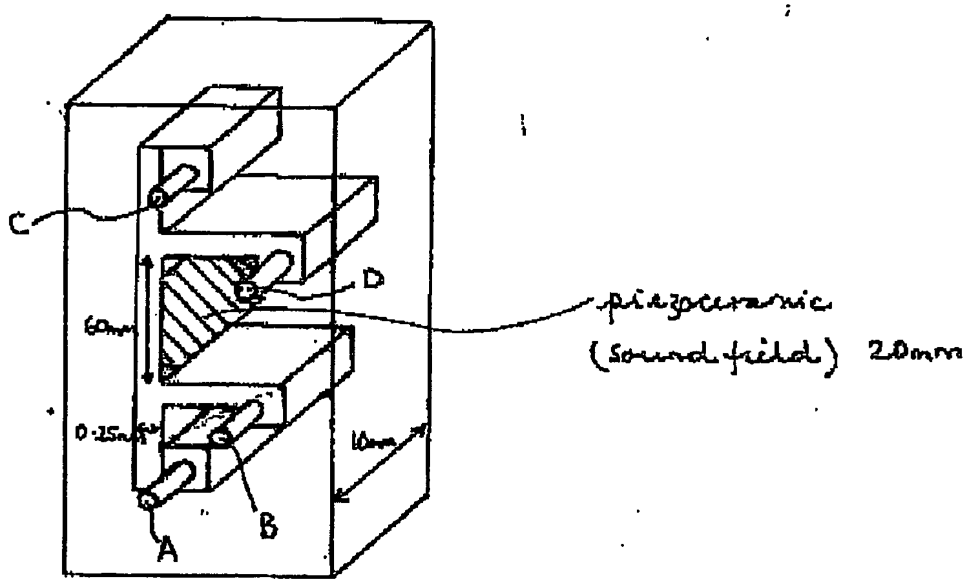


Figure 2 Diagrammatic representation of ultrasonic particle washing chamber.

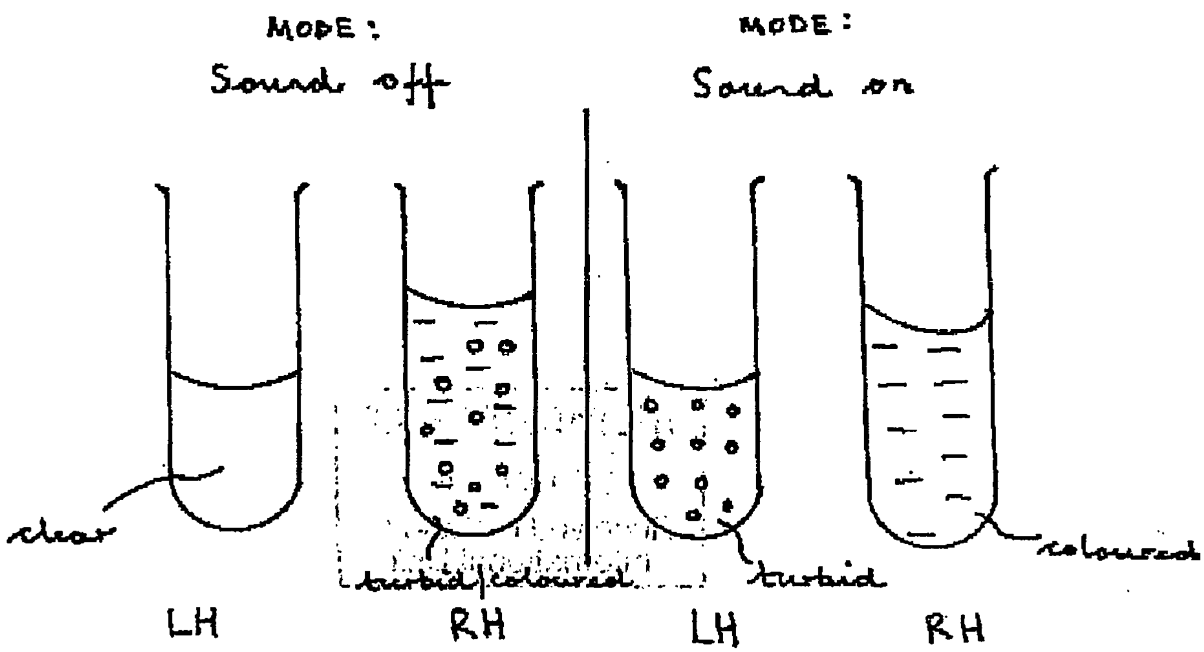


Figure 3 Fluid collected from outlets C (left hand, LH tubes) and D (right hand RH tubes). The dye solution is indicated by -, cells by o. As may be seen the left hand LH tube is free of dye or particles, while the right hand RH tube is turbid and coloured when the ultrasound is off. The left hand tube remains free of dye but is turbid because of cells while the right hand tube is not turbid but coloured when ultrasound is on.